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## Description

### DESCRIPTION OF THE BACKGROUND ART

Adenocarcinomas are carcinomas derived from glandular tissue or in which the tumor cells form recognizable glandular structures. There is increasing evidence suggesting that human adenocarcinomas characteristically express fucolipids representing blood group antigens and chemically related structures (Hakomori, Annual Reviews of Immunology, 2: 103-126, 1984). The blood group Y difucosylated hapten is structurally well defined and was first described in ovarian cyst glycoproteins (Lloyd, et al., Proceedings of the National Academy of Sciences, USA, 61: 1470-1477, 1968). Later, in its glycolipid form, this hapten was found to be present also in dog intestine (Smith, et al. Biochimica Biophysica Acta, 338: 171-179, 1975) and in human fetal intestine (Karlsson, et al., Journal of Biological Chemistry, 256: 3512-3524, 1981). More recently, a series of more complex glycolipids with dimeric and trimeric Y determinant structures have been characterized that are more abundant in human erythrocytes of blood group O than of blood group A individuals (Kannagi, et al., Journal of Biological Chemistry, 260: 6410-6415, 1985). This determinant has also been found to be present in human liver adenocarcinoma and as an oligosaccharide in the urine of lactating women.

Numerous monoclonal antibodies with anti-Y specific activity have been produced by immunizing mice with human gastric cancer, colon cancer, lung cancer, ovarian carcinoma, and human ovarian teratocarcinoma cells. The accumulation of antigens having the Y determinant has been reported in several human adenocarcinomas using the immunoperoxidase technique. The recently reported association between the Y determinant and the carcinoembrionic antigen enhances the relevancy of Y as a diagnostic marker in epithelial adenocarcinomas (Nichols, et al., Journal of Immunology, 135: 1911-1913, 1985).

Blaszczyk-Thurin et al. (The Journal of Biological Chemistry, Vol. 262, No. 1, pp. 372-379 (1987)) describe monoclonal antibody BR55-2 which was generated from mice immunized with MCF-7 human breast carcinoma cells. A series of glycolipids with Y and blood group B type 2 determinants were detected in human gastric adenocarcinoma cell line KATOIII with said monoclonal antibody and with a previously characterized anti-blood group B mAb PA83-52. The isolated antigens are structurally characterized by mass spectrometry of permethylated and permethylated-reduced derivatives and by proton NMR spectroscopy. The detection of a B type 2 determinant is the first chemical evidence for the presence of an autologous difucosyl blood group B type 2 antigen in human adenocarcinoma cells.

Although the presence of the Y determinant has been found in glycolipid associated with adenocarcinomas, it is unlikely that a monoclonal antibody which reacts solely with an epitope on the Y determinant would be clinically useful. This is because even though in a given tumor mass many of the malignant cells may express an antigen containing the Y determinant it is highly probable that a small, but significant, population of malignant cells will not express the Y determinant and, hence, would probably be refractory to immunotherapy centered on the administration of a Y-specific monoclonal antibody. It is these surviving cells which can enable a recurrence of the tumor mass. Thus, a need exists for a monoclonal antibody which is capable of reacting with an epitope present on multiple determinants of various antigens since such a monoclonal antibody would have far greater clinical efficacy by virtue of its ability to bind to many different populations of adenocarcinoma cells.

### SUMMARY OF THE INVENTION

The invention relates to the use of a monoclonal antibody for preparing a medicament as defined in claim 1. In particular, the present invention relates to the use of a monoclonal antibody for preparing a medicament for ameliorating malignant disease in an animal, said unlabeled or therapeutically labeled monoclonal antibodies reacting with determinants Y-6 and B-7-2.

It is a major advantage of the monoclonal antibodies used in the method for preparing a medicament that these monoclonal antibodies are capable, unlike the monoclonal antibodies of the prior art, of binding to an epitope which is present on multiple determinants. In so doing, the medicament for the therapeutic method utilizes these monoclonal antibodies being capable of binding to malignant cells which are expressing one or more of these determinants. In addition, since these determinants occur at a much greater frequ ncy on malignant cells than th y do on normal tissu th r is a much great r probability of binding occurring to a malignant c ll than to a normal cell. As a result of this fact, it is possibl to use conc ntrations of th monoclonal antibody which ar clinically effectiv , but pos minimal or no risk to host cell tissu .

## DETAILED DESCRIPTION

The present invention relates to the use of monoclonal antibodies with specificity for antigens indicative of adenocarcinomas as well as other tumors for preparing a medicament. These monoclonal antibodies are highly useful in a process for producing a medicament for the immunotherapy of tumors bearing these antigens.

The general method used for production of hybridomas secreting monoclonal antibodies is well known to those of ordinary skill in the art. Illustrative of the techniques utilized in the present invention are those described in Proceedings of the National Academy of Science, U.S.A., 75: 3405, (1978) and Koprowski, U.S. Patent No. 4,172,124 entitled "Method Of Producing Tumor Antibodies."

Briefly, BALB/c mice were immunized with cultured breast carcinoma cells (MCF 7) and later boosted with the same cell line. After 4 days, the animals were sacrificed and the spleen cells fused with the 653 variant of mouse myeloma P3X63 Ag8. Hybridomas were screened for antibody production and positive clones were tested for reactivity toward cell line MCF 7 and other cancer cell lines. In addition, class-switch variants were produced and isolated using known techniques (Steplewski, et al., Proceedings of the National Academy Of Science, U.S.A., 82: 8653, 1985).

The isolation of other hybridomas secreting monoclonal antibodies with the specificity of the above mentioned monoclonal antibodies can be accomplished by one of ordinary skill in the art by the technique of anti-idiotypic screening (Potocnjak, et al., Science, 215: 1637, 1982). An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the monoclonal antibody produced by the hybridoma of interest. These determinants are located in hypervariable region of the antibody. It is this region which binds to a given epitope and, thus, is responsible for the specificity of the antibody. The anti-idotypic antibody can be prepared by immunizing an animal with the monoclonal antibody of interest. The animal immunized will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants. By using the anti-idiotypic antibodies of the second animal, which are specific for the monoclonal antibodies produced by a single hybridoma which was used to immunize the second animal, it is now possible to identify other clones with exactly the same idiotype as the antibody of the hybridoma used for immunization.

Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody it is possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

The present invention is directed to the use of monoclonal antibodies, and hybridomas which produce them, which are reactive with carbohydrate determinants associated with glycolipid and glycoprotein molecules. It can easily be determined whether a monoclonal antibody has the requisite specificity by performing an antigen binding immunoassay such as that described in Thurin, et al. (Journal of Biological Chemistry, 262; 372, 1987).

Alternatively, since the inventors have characterized an epitopic moiety to which monoclonal antibodies having the specificity of those as mentioned before react (Thurin, et al, ibid), it is now a matter of routine skill to produce more hybridomas secreting monoclonal antibodies of identical epitopic specificity. The carbohydrate portion of the Y-6 or B-7-2 blood group determinants, which contain the epitope which binds the monoclonal antibodies can be purified from the major portion of the glycolipid by such techniques as ozonolysis (Sabesan, et al., Canadian Journal Of Chemistry, 62: 1034, 1984) or by specific enzymatic hydrolysis as with endoglycerocerami dase (Hito, et al., Journal Of Biological Chemistry, 262: 14278, 1986). Thus, additional hybridomas secreting monoclonal antibodies having the specificity of monoclonal antibodies produced by cell line ATCC HB 9324 or ATCC HB 9347 can be produced, for example, by coupling this epitope to an inert or immunogenic carrier molecule, such as KLH, to present the epitope in immunogenic form. (Hudson & Hay, Practical Immunology, p. 5-8, Blackwell Scientific Publications, 1980). In this manner, animals can be first immunized with whole Y-6 glycolipid, or cellular fractions enriched in Y-6 glycolipid, for initial sensitization of the animal followed by the conjugated epitope for purified antigen above in the booster immunization to stimulate outgrowth of the preferred B-cell clones.

Alternatively, one could initially immunize with one determinant, such as Y-6, and then boost with a different determinant, such as B-7-2, since the epitope reactive with the monoclonal antibodies is present on both of these blood group determinants. In any event, since the epitopic specificity of the monoclonal antibodies has been clearly defined (Thurin, et al., Journal of Biological Chemistry, 262; 372, 1987), it is possible to greatly restrict the repertoire of responder B-cell clones which are present for hybridoma fusion and thereby avoid undue experimentation in isolating hybridomas of the desired specificity. After fusion, the hybridomas are screened using the epitope and free carrier to select those clones producing monoclonal

antibodies which ar specific for this epitop .

While the us of monoclonal antibody from a foreign donor species in a differ nt host recipient species is usually uncomplicated, a potential problem which may aris is th appearanc of an adv rs immunological respons by the host to antigenic determinants pres nt on th donor antibody. In some instances, this adverse response can be so severe as to curtail the in vivo use of the donor antibody in the host. Further, the adverse host response may serve to hinder the malignancy-suppressing efficacy of the donor antibody. One way in which it is possible to circumvent the likelihood of an adverse immune response occurring in the host is by using chimeric antibodies (Sun, et al., Hybridoma, 5 (Supplement 1): S17, 1986; Oi et al., Bio Techniques, 4 (3): 214, 1986). Chimeric antibodies are antibodies in which the various domains of the antibodies heavy and light chains are coded for by DNA from more than one species. Typically, a chimeric antibody will comprise the variable domains of the heavy ($V_H$) and light ($V_L$) chains derived from the donor species producing the antibody of desired antigenic specificity and the constant antibody domains of the heavy ($C_H$) and light ($C_L$) chains derived from the host recipient species. It is believed that by reducing the exposure of the host immune system to the antigenic determinants of the donor antibody domains, especially those in the $C_H$ region, the possibility of an adverse immunological response occurring in the recipient species will be reduced. Thus, for example, it is possible to produce a chimeric antibody for in vivo clinical use in humans which comprises mouse $V_H$ and $V_L$ domains coded for by DNA isolated from ATCC HB 9324 or ATCC HB 9347 and $C_H$ and $C_L$ domains coded for a DNA isolated from a human cell.

Under certain circumstances, monoclonal antibodies of one isotype might be more preferably than those of another in terms of their diagnostic or therapeutic efficacy. For example, it is known that mouse monoclonal antibodies of isotype gamma-2a and gamma-3 are generally more effective in inhibiting the grown of tumors than is the gamma-1 isotype. This differential efficacy is thought to be due to the ability of the gamma-2a and gamma-3 isotypes to more actively participate in the cytolytic destruction of tumor cells. Particular isotypes of a monoclonal antibody can be prepared either directly, by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class-switch variants (Steplewski, et al., Proceedings of the National Academy of Science, U.S.A., 82: 8653, 1985; Spira, et al., Journal of Immunological Methods, 74: 307, 1984). Thus, the monoclonal antibodies would include class-switch variants having the specificity of monoclonal antibody BR55-2 which is produced by ATCC HB 9324 or BR55-2-S2a which is produced by ATCC HB 9347.

When the monoclonal antibodies are used in the form of fragments, such as, for example, Fab and F-$(ab')_2$, and especially when these fragments are therapeutically labeled, any isotype can be used since tumor inhibition in these situations is no longer dependent upon complement-mediated cytolytic destruction of the tumor cells.

The monoclonal antibodies can be used in a process for producing a medicament for any animal in which it is desirable to administer in vitro or in vivo immunotherapy. The term "animal" as used herein is meant to include both humans as well as non-humans.

The term "antibody" as used in this invention is meant to include intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding the epitopic determinant.

The monoclonal antibodies are suited for use in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the monoclonal antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize monoclonal antibodies are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay.

The monoclonal antibodies can be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibody, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, chemiluminescent compounds, and bioluminescent compounds. Those of ordinary skill in th art will know of oth r suitabl lab ls for binding to th monoclonal antibody, or will be abl to ascertain such, using routin exp rim ntation. Furthermor , th binding of thes labels to the monoclonal antibody of the invention can b don using standard techniques common to those of ordinary skill in the art.

The adenocarcinoma-associated antigen which is bound by the monoclonal antibodies may be present in biological fluids and tissues. Any sample containing a detectable amount of adenocarcinoma-associated antigen can be used. Normally, a sample is a liquid such as urine, saliva, cerebrospinal fluid, blood, serum and the like, or a solid or semi-solid such as tissues, feces, and the like.

As used in this invention, the term "epitope" is meant to include any determinant capable of specific interaction with the monoclonal antibodies of the invention. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

The monoclonal antibodies can be used to monitor the course of malignant disease in an individual. Thus, by measuring the increase or decrease in the size or number of malignant sites, or changes in the concentration of antigen shed into various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the malignancy is effective.

The medicament obtained in accordance with the present invention can also be used, alone or in combination with effector cells, for immunotherapy in an animal having a tumor which expresses adenocarcinoma-associated difucosyl blood group antigens with epitopes reactive with the monoclonal antibodies of the invention. When used in this manner, the dosage of monoclonal antibody can vary from 10 mg/m$^2$ to 2000 mg/m$^2$. The term "therapeutically effective" means that the amount of monoclonal antibody used is of sufficient quantity to ameliorate the cause of disease due to the malignacy.

When used for immunotherapy, the monoclonal antibodies may be unlabeled or labeled with a therapeutic agent. These agents can be coupled either directly or indirectly to the monoclonal antibodies. One example of indirect coupling is by use of a spacer moiety. These spacer moieties, in turn, can be either insoluble or soluble (Diener, et al., Science, 231: 148, 1986) and can be selected to enable drug release from the monoclonal antibody molecule at the target site. Examples of therapeutic agents which can be coupled to the monoclonal antibodies for immunotherapy are drugs, radioisotopes, immunomodulators, lectins and toxins.

The drugs which can be conjugated to the monoclonal antibodies include non-proteinaceous as well as proteinaceous drugs. The term "non-proteinaceous drugs" encompasses compounds which are classically referred to as drugs such as for example, mitomycin C, daunorubicin, and vinblastine.

The proteinaceous drugs with which the monoclonal antibodies can be labeled include immunomodulators and other biological response modifiers. The term "biological response modifiers" is meant to encompass substances which are involved in modifying the immune response in such a manner as to enhance the destruction of the tumor cells bearing the difucosyl blood group antigen for which the monoclonal antibodies are specific. Examples of immune response modifiers include such compounds as lymphokines. Examples of lymphokines include tumor necrosis factor, interleukins 1, 2, and 3, lymphotoxin, macrophage activating factor, migration inhibition factor, colony stimulating factor and interferon. Interferons with which the monoclonal antibodies of the invention can be labeled include alpha-interferon, beta-interferon, and gamma-interferon and their subtypes.

In using radioisotopically conjugated monoclonal antibodies in the method for producing a medicament for immunotherapy certain isotypes may be more preferable than others depending on such factors as tumor distribution and mass as well as isotype stability and emission. If desired, the tumor distribution and mass can be evaluated by in vivo diagnostic techniques. Depending on the type of malignancy present some emitters may be preferable to others. In general, alpha and beta particle-emitting radioisotopes are preferred in immunotherapy. For example, if an animal has solid tumor foci a high energy beta emitter capable of penetrating several millimeters of tissue, such as $^{90}$Y, may be preferable. On the other hand if the malignancy consists of single target cells, as in the case of leukemia, a short range, high energy alpha emitter such as $^{212}$Bi may be preferred. Examples of radioisotopes which can be bound to the monoclonal antibodies for therapeutic purposes are $^{125}$I, $^{131}$I, $^{90}$Y, $^{67}$Cu, $^{212}$Bi, $^{211}$At, $^{212}$Pb, $^{47}$Sc, $^{109}$Pd and $^{188}$Re.

Lectins are proteins, usually isolated from plant material, which bind to specific sugar moieties. Many lectins are also able to agglutinate cells and stimulate lymphocytes. However, ricin is a toxic lectin which has been used immunotherapeutically. This is accomplished by binding the alpha-peptide chain of ricin, which is responsible for toxicity, to the antibody molecule to enable site specific delivery of the toxic effect.

Toxins are poisonous substances produced by plants, animals, or microorganisms that, in sufficient dose, are often lethal. Diphtheria toxin is a substance produced by Corynebacterium diphtheriae which can be used in this manner. This toxin consists of an alpha and beta subunit which under proper conditions can be separated. The toxic A component can be bound to antibody and used for site specific delivery to a tumor expressing the difucosyl antigens for which the monoclonal antibodies are specific.

Other therapeutic agents which can be coupled to the monoclonal antibodies are known, or can be easily ascertained, by those of ordinary skill in the art.

The medicament containing labelled or unlabelled monoclonal antibodies can also be used in combination with therapeutic agents such as thos described supra. Especially pref rred are therapeutic combinations comprising the medicam nt pr pared according to the invention and immunomodulators and oth r biological response modifiers.

Thus, for example, the medicament prepared according to the invention can be used in combination with alpha-interferon. This treatment modality enhances monoclonal antibody targeting of carcinomas by increasing the expression of monoclonal antibody reactive antigen by the carcinoma cells (Greiner, et al., Science, 235:895, 1987). Alternatively, the medicament containing the monoclonal antibody prepared according to the invention could be used, for example, in combination with gamma-interferon to thereby activate and increase the expression of Fc receptors by effector cells which, in turn, results in an enhanced binding of the monoclonal antibody to the effector cell and killing of target tumor cells. Those of skill in the art will be able to select from the various biological response modifiers to create a desired effector function which enhances the efficacy of the medicament containing the monoclonal antibody.

When the medicament containing the monoclonal antibody is used in combination with various therapeutic agents, such as those described herein, the administration of the medicament and the therapeutic agent usually occurs substantially contemporaneously. The term "substantially contemporaneously" means that the monoclonal antibody and the therapeutic agent are administered reasonably close together with respect to time. Usually, it is preferred to administer the therapeutic agent before the medicament containing the monoclonal antibody. For example, the therapeutic agent can be administered 1 to 6 days before the medicament containing the monoclonal antibody. The administration of the therapeutic agent can be, daily or at any other interval depending upon such factors, for example, as the nature of the tumor, the condition of the patient and half-life of the agent.

Using the medicament containing the monoclonal antibodies it is possible to design therapies combining all of the characteristics described herein. For example, in a given situation it may be desirable to administer a therapeutic agent, or agents, prior to the administration of the medicament containing the monoclonal antibodies in combination with effector cells and the same, or different, therapeutic agent or agents. For example, it may be desirable to treat patients with adenocarcinoma by first administering gamma-interferon and interleukin-2 daily for 3 to 5 days, and on day 5 administer the medicament containing the monoclonal antibody in combination with effector cells as well as gamma-interferon, and interleukin-2.

It is also possible to utilize liposomes with the medicament containing the monoclonal antibodies in their membrane to specifically deliver the liposome to the area of the tumor expressing difucosyl blood group antigens Y-6 or B-7-2. These liposomes can be produced such that they contain, in addition to the medicament such immunotherapeutic agents as those described above which would then be released at the tumor site (Wolff, et al., Biochemica et Biophysica Acta, 802: 259, 1984).

The dosage ranges for the administration of the medicament containing the monoclonal antibodies are those large enough to produce the desired effect in which the symptoms of the difucosyl expressing tumor are ameliorated. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counter indications, immune tolerance or similar conditions. Dosage can vary from 0.1 $mg/m^2$ to 2000 $mg/m^2$, preferably 0.1 $mg/m^2$ to 500 $mg/m^2$/dose, in one or more dose administrations daily, for one or several days. Generally, when the medicament containing the monoclonal antibodies are administered conjugated with therapeutic agents lower dosages, such as those used for in vivo immunodiagnostic imaging, can be used.

The medicament containing the monoclonal antibodies can be administered parenterally by injection or by gradual perfusion over time. The monoclonal antibodies of the invention can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally, alone or in combination with effector cells.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as, olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parent ral vehicles includ sodium chloride solution, Ringer's dextros , dextros and sodium chlorid , lactat d Ringer's, or fixed oils. Intravenous vehicles includ fluid and nutri nt replenish rs, electrolyte replenish rs, such as those based on Ring r's dextrose, and the like. Preservatives and other additives may also b pres nt such as, for xampl , antimicrobials, anti-oxidants, chelating agents, and in rt gases and th lik .

The invention relates to a method for preparing a medicament or pharmaceutical composition comprising monoclonal antibodies as described the medicament being used for therapy of tumors expressing the difucosyl blood group antigens Y-6 and B-7-2 or active with said monoclonal antibodies.

Monoclonal antibody can be utilized in the present invention. BR55-2 is obtained from, or has the identifying characteristics of, an antibody obtained from the cell line having ATCC accession number HB 9324. BR55-2-S2a is obtained from, or has the identifying characteristics of, an antibody obtained from the cell line having ATCC accession number HB 9347. These cell lines were placed on deposit for 30 years at the American Type Culture Collection (ATCC) in Rockville, Maryland prior to March 27, 1987.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

## EXAMPLE 1

### PREPARATION OF HYBRIDOMA CELL LINES PRODUCING MONOCLONAL ANTIBODIES TO ADENOCARCINOMA-ASSOCIATED ANTIGENS

#### A. Immunization And Production of Hybridomas

BALB/c mice were immunized intraperitoneally with $2x10^7$ cells of breast adenocarcinoma MCF-7 and boosted intravenously four weeks later with $1x10^6$ cells. Four days later, the animals were sacrificed and spleen cells fused with the 653 variant of mouse melanoma P3X63 Ag8. The growth, cloning and maintenance of the hybridomas produced was as described by Koprowski, et al. (Somatic Cell Genetics, 5: 957, 1979). Monoclonal antibodies produced by the various hybridomas were screened for antibody production and positive clones were further screened for reactivity towards cell line MCF-7 and no reactivity toward human melanoma cell line WM164 and others. Selected cultures were then cloned using the limiting dilution technique. Hybridoma class-switch variants were produced using the procedures described by Steplewski, et al. (Proceedings of the National Academy of Sciences, U.S.A., 82: 8653, 1985). The characterization of the epitopic specificity of BR 55-2 and BR 55-2-S2a has been described (Thurin, et al., Journal Of Biological Chemistry, 262: 372, 1987). Monoclonal antibodies were purified for clinical testing according to Sears, et al. (THE LANCET, 762, April 3, 1982).

#### B. Glycolipids

The purification and characterization of the various blood group antigens was performed essentially using the techniques described in Thurin, et al. (Journal Of Biological Chemistry, 260: 14556, 1985)

## EXAMPLE 2

### IN VIVO CLINICAL TRIALS IN HUMANS USING BR55-2-S2a

Patients with terminal gastrointestinal cancer which presented with recurrence, metastasis or unresectable tumors were included in the study if they were less than 75 year old, with a Karnosky index greater than 60, with a life expectancy of more than 3 months and when the primary tumor type was clearly identified as a gastrointestinal tract adenocarcinoma using biopsy material.

Patient tumors, or when available, metastisis biopsy specimens, were studied for antigenic expression using monoclonal antibodies BR55-2-S2a, CO 19-9, Ga 73-3 and CO 17-1A. Based on these individual immunohistochemical results, a cocktail of monoclonal antibodies was administered to the patient.

In treating the patients, autologous peripheral blood mononuclear cells were obtained by leukophoresis, through a routine procedure using an IBM 2997 blood cell separator to yield a total number of $1x10^9$ to $1x10^{10}$ mononuclear cells. The cells obtained by leukophoresis were collected in a sterile plastic bag with a total volume of 160 to 200 ml. The monoclonal antibodies (150-200mg of each) for which the patient tumor was reactive were then injected directly into the leukobag and allowed to incubate at room temperature at one hour, with gentle shaking every 15 minutes. In so doing, the monoclonal antibodies were able to bind to effector cells via Fc receptors on the surface of the cells and thereby act as a vector for effector cell targeting to the tumor. Sampling of free monoclonal antibody in the supernatant from the leukobag showed that, on the average, 40% of the amount of injected antibody bound to the autologous cells. After this incubation period, the mixture of autologous cells and free monoclonal antibodies in the leukobag were

reinfused into a peripheral vein, or into th hepatic artery, over a period of 2 to 3 hours. After infusion, th lin was k pt in the blood v ss l in order to treat possible delayed sid affects. Patients wer car fully checked for blood pressur , pulse, chills, skin rash, bronchio-spasm or any other suspect clinical signs during infusion and again 24 hours ther after. Th r sult of Phase I clinical trials are shown in Table 1.

**TABLE 1**

**RESULTS OF PHASE I CLINICAL TRIALS USING BR55-2-S2a[a]**

| CANCER DIAGNOSIS | | PATIENT | MONTHS STABLE IN RESPONSE TO THERAPY[b] |
|---|---|---|---|
| ORIGIN | METASTASES | | |
| colon | liver | 9 | |
| | | 14 | 6 |
| | | 25 | 6, + |
| | | 39 | 7, + |
| | lung | 12 | 0 |
| | | 26 | 8, + |
| | liver, pleura | 34 | 0 |
| | liver, lung | 40 | 7,+ |
| | skin | 36 | 0 |
| | local lymph nodes | 43 | 6 |
| breast | bone, skin | 2 | tumor regression |
| | pleura, skin | 27 | 5,+ |
| rectal | liver | 10 | 2 |
| | | 15 | 2 |
| | | 17 | 5 |
| | | 30 | 15 |
| | | 33 | 8,+ |
| gastric | local recurrence | 18 | 3 |
| | lung | 38 | 7,+ |
| pancreas | unresectable liver | 20 | 9 |
| | | 22 | 8 |
| | | 31 | 2 |
| | liver, lymph nodes | 32 | 6 |
| | lymph nodes | 35 | 1 |

a gamma-2A variant in admixture with other monoclonal antibodies and effector cells

b "+" denotes that patient was still stable at time of data

**compilation**

The cancer patients treated with B55-2-S2a had primary tumor foci of the colon (10), breast (2), rectum (5), gastric tract (2) and pancreas (5). As noted, all patients had some degree of metastases. This data is further summarized in Table 2.

TABLE 2

CLINICAL SUMMARY

| TUMOR ORIGIN | NUMBER OF PATIENTS | RESPONSE | | |
|---|---|---|---|---|
| | | NONE | TEMPORARY STABILITY | CONTINUING STABILITY |
| colon | 10 | 4 | 2 | 4 |
| breast | 2 | — | — | 4[a] |
| rectal | 5 | — | 4 | 1 |
| gastric | 2 | — | 1 | 1 |
| pancreas | 5 | — | 5 | — |
| total | 24 | 4 | 12 | 8 |

[a] one patient had tumor regression

As shown here, of the 24 patients in this study receiving BR55-2-S2a, 4 apparently did not respond to the monoclonal antibody therapy. Of the 20 patients responding favorably to therapy, 12 were temporarily stable for an average of 5.3 months. The eight remaining patients who responded to therapy were still stable at the time the data was tabulated. Among this group of patients showing continued stability, the average response to therapy was 6.9 months and included 1 patient who had experienced tumor regression.

**Claims**

1. The use of a monoclonal antibody having the epitopic specificity of a monoclonal antibody produced by a hybridoma deposited under ATCC Accession number HB 9324 or HB 9347, in a process for producing a medicament for suppressing tumors which express difucosyl blood group antigens, said antigens being reactive with the monoclonal antibody produced by said hybridoma.

2. The use of the monoclonal antibody of claim 1, wherein said monoclonal antibody is labeled by a therapeutic label selected from the group consisting of a radioisotope, a drug, an immunomodulator, a biological r spons modifi r, a l ctin and a toxin.

3. The use of the monoclonal antibody of claim 1 or 2, wher in said monoclonal antibody is labeled by a detectabl lab l, in particular a d t ctabl lab l s l ct d from th group consisting of
   - radioisotope and/or paramagnetic labels and/or from the group consisting of

- radioisotope, fluorescent compounds, chemiluminesc nt compounds, bioluminescent compounds and enzym s.

**Pat ntansprüche**

1. Verwendung eines monoklonalen Antikörpers mit der Epitopspezifität eines monoklonalen Antikörpers, der von einem unter der ATCC-Hinterlegungsnummer HB 9324 oder HB 9347 hinterlegten Hybridom gebildet wird, in einem Verfahren zur Herstellung eines Arzneimittels zur Unterdrückung von Tumoren, die Difucosyl-Blutgruppenantigene exprimieren, wobei die Antigene mit dem durch das Hybridom gebildeten monoklonalen Antikörper reaktiv sind.

2. Verwendung des monoklonalen Antikörpers nach Anspruch 1, wobei der monoklonale Antikörper mit einer therapeutischen Markierung markiert ist, die aus der Gruppe ein Radioisotop, ein Arzneistoff, ein Immunomodulator, ein Modifikationsmittel der biologischen Reaktion, ein Lectin und ein Toxin ausgewählt ist.

3. Monoklonaler Antikörper nach Anspruch 1 oder 2, wobei der monoklonale Antikörper mit einer nachweisbaren Markierung markiert ist, insbesondere mit einer nachweisbaren Markierung, die aus der Gruppe
   - radioisotopische und/oder paramagnetische Markierungen und/oder aus der Gruppe
   - Radioisotop, fluoreszierende Verbindungen, chemilumineszierende Verbindungen, biolumineszierende Verbindungen und Enzyme

   ausgewählt ist.

**Revendications**

1. L'utilisation d'un anticorps monoclonal ayant la spécificité épitopique d'un anticorps monoclonal produit par un hybridome déposé sous le numéro d'accession ATCC HB 9324 ou HB 9347, dans un processus de production d'un médicament pour supprimer des tumeurs qui expriment des antigènes difucosyle de groupe sanguin, ces antigènes étant réactifs avec l'anticorps monoclonal produit par ledit hybridome.

2. L'utilisation de l'anticorps monoclonal de la revendication 1, où cet anticorps monoclonal est marqué par un marqueur thérapeutique choisi dans le groupe formé par un radioisotope, un médicament, un modificateur de réponse biologique, une lectine et une toxine.

3. L'utilisation de l'anticorps monoclonal de la revendication 1 ou 2, où cet anticorps monoclonal est marqué par un marqueur détectable, en particulier un marqueur détectable choisi dans le groupe formé par
   - un radioisotope et/ou des marqueurs paramagnétiques, et/ou dans le groupe formé par
   - un radioisotope, des composés fluorescents, des composés chimioluminescents, des composés bioluminescents et des enzymes.